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# Structure, oligosaccharide structures, and posttranslationally modified sites of the nicotinic acetylcholine receptor

**(glycosylation/ion channels/liquid secondary ion mass spectrometry/mass spectrometry/multichannel array detection)**

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**ABSTRACT** **Using mass spectrometry, we have examined the transmembrane topography of the nicotinic acetylcholine receptor, a five-subunit glycosylated protein complex that forms a gated ion channel in the neuromuscular junction. The primary sequences of the four polypeptide chains making up the acetylcholine receptor from *Torpedo californica* contain many possible sites for glycosylation or phosphorylation. We have used liquid secondary ion mass spectrometry to identify posttranslationally modified residues and to determine the intact oligosaccharide structures of the carbohydrate present on the acetylcholine receptor. Asparagine-143 of the α subunit (in consensus numbering) is shown to be glycosylated with high-mannose oligosaccharide. Asparagine-453 of the γ subunit is not glycosylated, a fact that bears on the question of the orientations of putative transmembranous helices M3, MA, and M4. The structures of the six major acetylcholine receptor oligosaccharides are determined: the major components (70%) are of the high-mannose type, with bi-, tri-, and tetraantennary complex oligosaccharides making up ≈22 mol% of the total carbohydrate. This application of a multichannel array detector mass spectrometer provided a breakthrough in sensitivity that allowed us to identify the site of attachment of, and the sequence of, oligosaccharides on a 300-kDa membrane protein from only 5 pmol of the isolated oligosaccharide.**

How the nicotinic acetylcholine receptor (AcChoR) mediates ion conductance in response to binding acetylcholine at the neuromuscular junction is of critical importance to understanding the function of the family of related neuroreceptors (1). The molecular structure of the AcChoR contains five similar subunits that surround the ion-conducting channel, shown to lie in the center (2, 3). While the primary structures of all four subunit types of the acetylcholine receptor from *Torpedo californica* are homologous from N to C termini (4), there is as yet no identification of all regions that are transmembranous. However, in each of the five subunits ($\alpha_2\beta\gamma\delta$), there are four hydrophobic stretches of 19–26 amino acids that are presumed to lie across the membrane (5). Site-directed mutagenesis shows that residues of the second helix, M2, determine aspects of the conductivity (6). On the basis of our x-ray diffraction evidence for oriented α-helices perpendicular to the membrane we proposed that the ion channel could be formed within a bundle of such α-helices. More polar surfaces could provide the lining of the pore. A fifth amphipathic α-helix, which may also be transmembranous (MA), is apparent from the AcChoR primary sequence. Our hypothesis that these helices could also contribute to the pore led to experiments designed to determine the peptide topography, especially in the region of MA, M4, and the C terminus. Our electron microscopic localization of peptide-specific antibodies on intact native tissue and on subcellular fractions (7) and experiments of Ratnam *et al.* (8) all indicate that certain regions between M3 and M4, and the C termini are cytoplasmic. However, related sequence families in the γ-aminobutyric acid and glycine receptors, which clearly seem to have similar structure to the AcChoR, do not contain an amphipathic helix like MA (9). This conjecture remains the strongest evidence that MA does not cross the membrane and so cannot form part of the channel. We designed a strategy that in principle can determine complete topography of any such membrane protein; we begin by reporting its application to unambiguous definition of posttranslationally modified sites that contain topographical information. On the common presumption that glycosylated sites in membrane proteins are extracellular, we sought to determine where these are located. One consensus site for N-glycosylation (Asn-Xaa-Ser/Thr) lies in the region between MA and M4 in the γ chain alone; glycosylation here, had it occurred, would serve to define topography in this critical region. We show here that this site does not carry polysaccharide, again leaving open the absolute determination of sidedness of the MA–M4 linking region. We also find that Asn-$143_{\alpha C}$‖ is glycosylated with high-mannose oligosaccharide, and we elucidate the structures of >90% of the oligosaccharides attached to the AcChoR. The methods we describe now provide a means of unambiguous resolution of AcChoR topography by using exogenous labels from within the membrane as well as from either side of the membrane.

## MATERIALS AND METHODS

**AcChoR Purification.** AcChoR was affinity purified from *T. californica* according to ref. 10 with minor changes: NaF (100 mM) was substituted for NaCl and protease inhibitors (20 μg of leupeptin per ml, 20 μg of antipain per ml, 20 units of trasylol per ml) were included to inhibit endogenous phosphorylases. In preparing the acetylcholine affinity column, AffiGel-10 derivatized with cystamine was used instead of AffiGel-401. Residual carbamoylcholine and cholate were removed by dialysis, and then the dialysis buffer was exchanged for $NH_4HCO_3$ (50 mM; pH 7.8).

AcChoR was reduced with dithiothreitol (10 mM) in $NH_4HCO_3$ (50 mM; pH 8.2–8.3) under $N_2$ for 2 hr at 37°C, the thiols were alkylated with 20 mM iodoacetate at room temperature for 1 hr, and then reagents were removed by dialysis against ammonium bicarbonate (50 mM; pH 7.8).

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Abbreviations: AcChoR, acetylcholine receptor; ABEE, *p*-aminobenzoic acid ethyl ester; LSIMS, liquid secondary ion mass spectrometry; PNGase F, peptide *N*-glycosidase.

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‖Residues with the subunit and "C" subscript refer to the consensus numbering scheme of Stroud and Finer-Moore (2); residues with only the subunit subscript are sequential in the primary sequence.

**Fractionation of AcChoR for Mass Spectrometry.** Tryptic and *Staphylococcus aureus* V8 digestions of the receptor (500 μg; 2 nmol) were carried out in ammonium bicarbonate (50 mM; pH 7.8) with 0.1% $NaDodSO_4$. After digestion, $NaDodSO_4$ was removed by precipitation with guanidine hydrochloride (11). Peptides and glycopeptides were separated by gel filtration on a G-50 column using 50 mM ammonium bicarbonate as eluent and monitoring at 210 and 280 nm. Aliquots of these fractions were acid hydrolyzed to release monosaccharides, which were then derivatized as trimethylsilyl methyl glycosides. Gas chromatographic analysis of the derivatives gave the monosaccharide composition and therefore indicated whether the oligosaccharides were of the high-mannose or complex types. Aliquots of the oligosaccharide-containing fractions were treated with peptide *N*-glycosidase F (PNGase F), which cleaves high-mannose, hybrid, and complex glycans from N-linked glycosylation sites, while converting asparagine to aspartic acid (12). Reverse-phase HPLC [on a Vydac column (C18, C4, and biphenyl), using water/0.1% trifluoroacetic acid and acetonitrile/0.09% trifluoroacetic acid as the aqueous and organic phases, respectively] of peptides before and after deglycosylation further fractionated the peptides for analysis by mass spectrometry.

Oligosaccharides were released from N-linked glycosylation sites by digestion of AcChoR (500 μg; 2 nmol) with PNGase F. In a modification of previously described methods (13, 14), AcChoR was digested in ammonium bicarbonate buffer (100 mM; pH 8.6) with 0.1% $NaDodSO_4$ and 0.6% Nonidet P-40 at 37°C for 18 hr. Analytical $NaDodSO_4$/PAGE (15) showed digestion to be complete. $NaDodSO_4$ was precipitated as described earlier, and oligosaccharides were separated from protein and residual detergent by passage through a disposable reverse-phase matrix (Sep-Pak C18 cartridge; Waters). The aqueous fraction, containing all released oligosaccharides, was either lyophilized directly or treated with neuraminidase before lyophilization, and then derivatized according to a modification of the method described by Wang *et al.* (16) in which *p*-aminobenzoic acid ethyl ester (ABEE) (16.5 mg) and sodium cyanoborohydride (3.5 mg) were added to the sample in 100 μl of methanol/acetic acid (9:1, vol/vol), and the solution was incubated at 80°C for 40 min. After cooling, 500 μl of chloroform was added and the mixture was extracted twice with water (500 μl). The combined water layers were lyophilized and the derivatized oligosaccharides were purified by HPLC. An aminopropyl column was used for desialylated oligosaccharides, with a gradient of 80–40% acetonitrile over 40 min, while monitoring at 320 nm. Sialylated structures were purified on C18 or C4 reverse-phase columns using water and acetonitrile as the aqueous and organic phases and incorporating the following elution program: 0–10 min, 100% water; 10–70 min, 0–60% acetonitrile. The eluent was monitored at 320 nm.

**Mass Spectrometry.** All peptide fractions collected from each reverse-phase HPLC analysis were subjected to liquid secondary ion mass spectrometry (LSIMS) in the positive ion mode (14). Experimentally derived molecular weights, together with sequences obtained from peptide fragmentation, were used to assign peptides to regions within one of the four receptor subunits. The expected masses of peptides produced by proteolytic cleavage were calculated from the cDNA sequence (4).

Mass spectra of derivatized oligosaccharides were recorded in the positive-ion mode to obtain molecular weights and purity. In this mode, fragmentation of this derivative is minimal, allowing identification of the molecular ion at high sensitivity (14). Negative-ion spectra were recorded to obtain fragmentation and to determine oligosaccharide sequences (14).

All mass spectra of peptides and oligosaccharides, unless otherwise stated, were recorded with a Kratos MS 50-S double-focusing mass spectrometer, equipped with a high-field magnet, a LSIMS source (17, 18), and a postacceleration detector (10 keV).

In pioneering experiments in which multichannel array detection was used, negative ion spectra were recorded with a Kratos Concept. The characteristics of the multichannel electrooptical array detection system used in these studies have been reported (19). Negative-ion LSIMS spectra were obtained by computer-controlled acquisition from the array detector in sequential 4% mass segments (20).

## RESULTS

**Peptide Mapping of AcChoR Subunits.** AcChoR peptides identified by mass spectrometry are listed in Fig. 1. The regions of the AcChoR that were mapped by mass spectrometry are heavy-lined on the predicted secondary structure diagram of the consensus subunit in Fig. 1. The N-terminal peptides, the cytoplasmic loops downstream from M3, and the proposed amphipathic helical domains (MA) were most readily digested and mapped by mass spectrometry, reflecting an increased number of trypsin/V8 cleavage sites in these regions. Residues 81–180 and the residues toward the C terminus from 490 did have cleavage sites but were generally resistant to cleavage. The hydrophobic putative transmembrane domains lack V8 and trypsin cleavage sites and are thus not yet amenable to mass spectrometry analysis.

A reverse-phase HPLC chromatogram of one of the peptide fractions clearly showed an absence of one peak upon deglycosylation with PNGase F (data not shown). One of the peptides in the peak fraction after deglycosylation had a molecular weight $MH^+$ = 1980 (nominal mass), which was not present in the mass spectrum of this HPLC fraction prior to deglycosylation (Fig. 2). This molecular weight corresponds to the peptide Ile-Ile-Val-Thr-His-Phe-Pro-Phe-Asp-Gln-Gln-Asp-Cys-Thr-Met-Lys, which is the expected product of PNGase F digestion of the glycosylated peptide $132–147_{\alpha C}$; $130–145_{\alpha}$. This demonstrates that Asn-$143_{\alpha C}$ is glycosylated in the native state. Moreover, GC analysis of the trimethylsilyl methyl glycosides of monosaccharides released from this glycopeptide demonstrated that only mannose and *N*-acetylglucosamine were present, indicating that a high-mannose structure is attached at this site.

Another potential glycosylation site that was mapped by mass spectrometry was Asn-$453_{\gamma C}$ ($431_{\gamma}$). A molecular ion with $MH^+$ = 1181 was found, the mass of which matches an unglycosylated peptide from the γ subunit, Ser-Thr-Lys-Glu-Gln-Asn-Asp-Ser-His-Ser-Glu. The equivalent peptide with aspartate substituted for asparagine was not found in any PNGase-treated sample, demonstrating that the γ subunit is not glycosylated at Asn-$453_{\gamma C}$.

All of the seven putative sites for phosphorylation by cAMP-dependent protein kinase, protein kinase C, or tyrosine kinase were mapped in these experiments, and no peptides containing phosphoserine, phosphotyrosine, or phosphothreonine were detected.

**Oligosaccharide Analysis.** Mass spectrometric analyses showed the major fraction of receptor oligosaccharides (≈70% as judged by integrated absorbance at 320 nm during HPLC purifications) to be high-mannose structures with compositions $Man_8GlcNAc_2$-ABEE and $Man_9GlcNAc_2$-ABEE, as judged by positive-ion mass spectra, which showed protonated molecular ions at $MH^+$ = 1870 and $MH^+$ = 2032, respectively. Full details of the fragmentation observed are provided in Fig. 3, which shows the negative-ion mass spectrum recorded using ≈10 ng (5 pmol) of $Man_8GlcNAc_2$-ABEE using computer-controlled mass window stepping and multichannel array detection (20).

A

| α ◀ | $\alpha_C$ | β ◁ | $\beta_C$ |
|---|---|---|---|
| Thr5 -Glu13 | 5 -14 | Ser1 -Glu45 | 1 -47 |
| Leu56 -Arg64 | 58 -66 | Ser126-Lys131 | 128-133 |
| Trp67 -Lys76 | 69 -78 | Val156-Arg168 | 158-174 |
| Ile116-Lys125 | 118-127 | Glu172-Lys191 | 178-198 |
| Ile130-Lys155 | 132-157 | Ser308-Arg319 | 320-331 |
| Ser162-Glu172 | 171-187 | Ala351-Lys385 | 373-409 |
| Ile315-Lys380 | 333-443 | Phe421-Lys425 | 455-459 |
| Leu430-Gly437 | 493-500 | | |
| **γ ■** | $\gamma_C$ | **δ □** | $\delta_C$ |
| Lys10 -Arg18 | 10 -20 | Leu7 -Lys25 | 7 -25 |
| Gly74 -Arg79 | 76 -81 | Leu278-Lys290 | 282-294 |
| Val156-Glu166 | 158-168 | Thr316-Lys327 | 320-331 |
| Asn181-Lys201 | 188-209 | Ile337-Arg342 | 341-346 |
| Lys346-Arg381 | 354-394 | Gln348-Arg395 | 352-402 |
| Asp405-Glu438 | 427-460 | Ile400-Glu407 | 408-423 |
| | | Ser421-Lys431 | 437-447 |

B

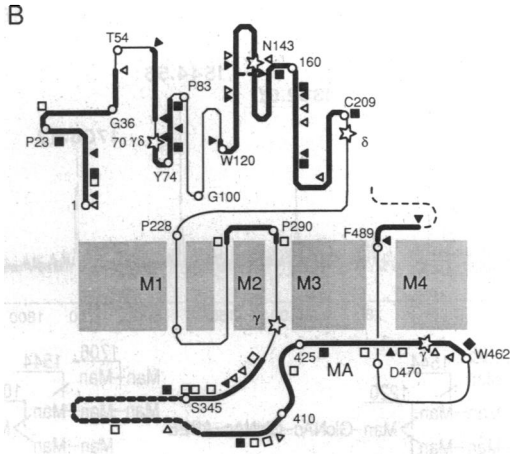



FIG. 1. AcChoR peptides identified by LSIMS. (*A*) Mapped peptides of each subunit are identified by both primary sequence numbering and consensus numbering (2). (*B*) The consensus subunit secondary structure model is used to illustrate the locations of the identified peptides. Subunit-specific cleavage sites are shown: ▲, α chain; △, β chain; ■, γ chain; □, δ chain. Putative glycosylation sites are denoted by stars, accompanied by the subunit symbol where the sites are not conserved in all subunits. Some of the conserved amino acids are included as reference points; they are denoted by the single-letter abbreviation and the consensus number.

Analysis of neuraminidase-treated samples showed the major species present in the remaining 30% of oligosaccharide to be nonfucosylated tri-, tetra-, and biantennary complex structures, with $MH^+ = 2155$, $MH^+ = 2520$, and $MH^+ = 1790$, respectively. A fourth component with $MH^+ = 2358$ had the composition $Man_3Gal_3GlcNAc_6$ as judged by molecular weight and monosaccharide analysis. Fragmentation observed in the negative-ion mode indicated a tri-antennary structure with a bisecting *N*-acetylglucosamine residue. All structures observed by mass spectrometry are detailed in Fig. 4.

In our earlier experiments, sialic acid residues were removed from all oligosaccharides by neuraminidase treatment to simplify oligosaccharide analysis. More recently, we have prepared and purified sialo-ABEE derivatives from receptor oligosaccharides. Fig. 5 shows a spectrum obtained by using 1 μg of an ABEE-derivatized trisialo-triantennary structure purified by reverse-phase HPLC. The sensitivity achieved with this derivatization is roughly 10- to 20-fold greater than that attained in the analysis of underivatized sialo-oligosaccharides and is due to the introduction of the hydrophobic group, which increases the surface activity of the oligosaccharide in the liquid matrix during mass spectrometry analysis (21). The elution positions of oligosaccharides in the reverse phase HPLC chromatograms of sialo-ABEE structures indicate that all of the complex oligosaccharides are highly sialylated.

## DISCUSSION

**Glycosylation.** We have determined the structures of the major oligosaccharides in AcChoR from *T. californica*; two are of the high-mannose type, and four are complex structures. High-mannose oligosaccharides are attached to every α chain at Asn-$143_{\alpha C}$ ($141_\alpha$). All four subunits were shown to be glycosylated (22) by oligosaccharides of either the high-mannose or complex type. Nomoto *et al.* (23) demonstrated that high-mannose oligosaccharides are present on all subunits, with complex oligosaccharides present only on the γ and δ chains. No oligosaccharide structures containing fucose were found by mass spectrometry. This finding is in contrast to the results of Nomoto *et al.* (23), who indicate fucosylated chitobiose core on a proportion of bi-, tri-, and tetraantennary structures. Their evidence, however, is primarily based on a comparison of HPLC retention times with those of putative standards, which clearly is an inadequate sole criterion for oligosaccharide identification.

Primary sequences of the four subunits reveal one possible site for N-linked glycosylation (consensus sequence is Asn-Xaa-Ser/Thr) on α, one on β, four on γ, and three on δ, as shown in Fig. 1. Because the α chain is glycosylated, and because the primary sequence shows only one site for N-glycosylation, this site was assumed to be the point of carbohydrate attachment. Nevertheless, the positive identification of glycosylated Asn-$143_{\alpha C}$ by mass spectrometry is

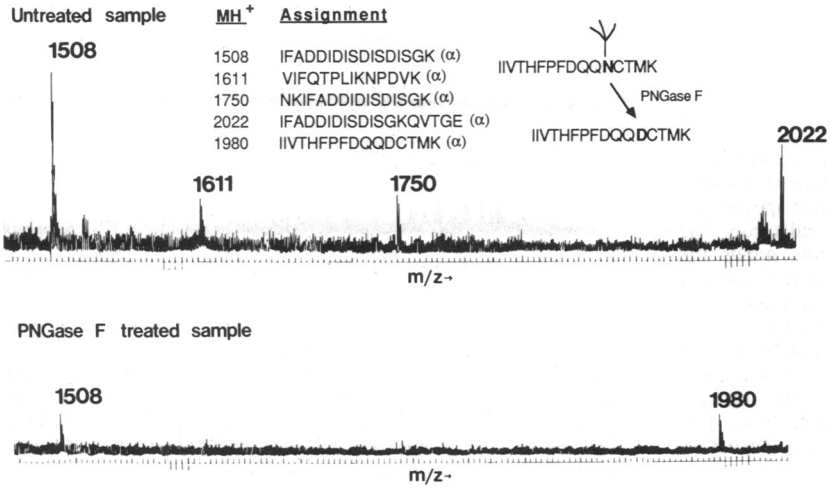



FIG. 2. The positive-ion LSIMS spectrum of the peptide fraction containing glycosylation site Asn-$143_{\alpha C}$ before (*Upper*) and after (*Lower*) PNGase F digestion. The molecular ion appearing at *m/z* 1980 (nominal mass) corresponds exactly to a peptide containing Asn-$143_{\alpha C}$, with the conversion of Asn-$143_{\alpha C}$ to aspartic acid after deglycosylation (see *Inset*). Amino acids are denoted by the single-letter code. (*Lower*) A poorer signal to noise ratio is observed and is probably due to traces of detergent that were included in the enzymatic digestion. Other molecular ions, observed at *m/z* 1508, 1611, 1750, and 2022, correspond to peptides from the α subunit, and their identities are also provided.

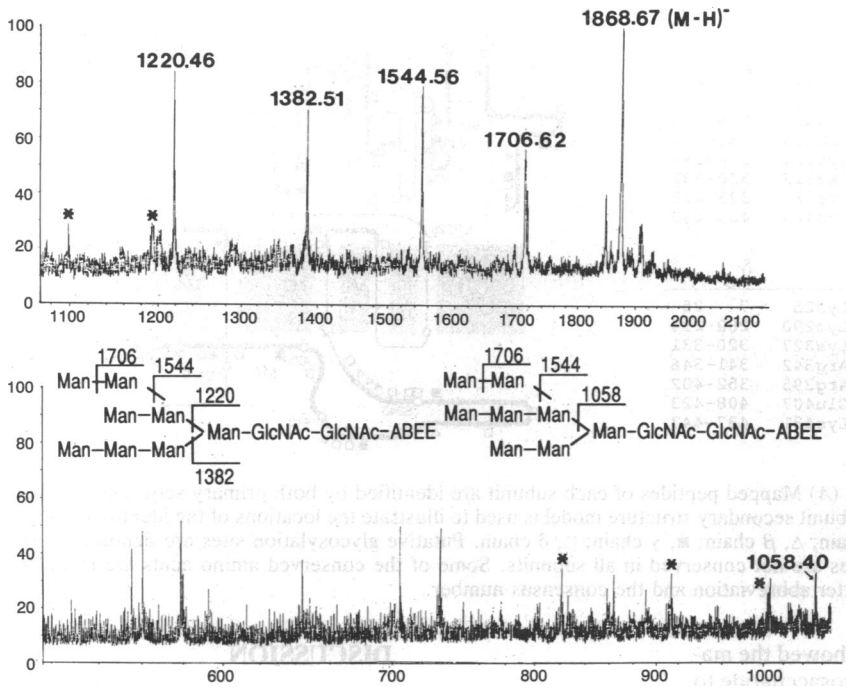



FIG. 3. The negative-ion LSIMS spectrum obtained on 10 ng (5 pmol) of $Man_8GlcNAc_2$-ABEE from AcChoR, using multichannel array detection in combination with computer-controlled mass window stepping. Losses of one, two, three, and four mannose units are prominent, which is in agreement with the structure predicted by the $^1H$ NMR work of Nomoto *et al.* (23). A weak fragment ion, corresponding to the loss of $Man_5$, is also detected well above the noise level (ions marked by asterisks arise from protonated glycerol matrix cluster ions, in which the sample was dissolved; ref. 16); this ion could arise from two bond cleavages or from the presence of a minor component of isomeric structure. No minor component was detected by $^1H$ NMR or without the use of the array system.

the first instance of assigning a glycosylation site to a specific amino acid on the acetylcholine receptor.

It has been postulated that differential glycosylation of the two $\alpha$ chains is responsible for what may be different affinities of competitive antagonists for the receptor (24, 25). This postulate was supported by peptide mapping experiments in which two V8 fragments of the $\alpha$ chain, an 18-kDa fragment and a 20-kDa fragment, were both believed to contain Asn-$143_{\alpha C}$, yet only one was glycosylated (26). However, Pedersen *et al.* (27) demonstrated that under certain conditions the 20-kDa V8 fragment is composed of two distinct 20-kDa fragments, the major component being Ser-173–Glu-335 (containing no glycosylation site), and the minor component being glycosylated Val-46–Glu-172. Our results demonstrate that all $\alpha$ chains have carbohydrate attached, since otherwise a non-glycosylated $\alpha$-peptide containing Asn-$143_{\alpha C}$ would have been detected by mass spectrometry even if it were a minor component. The possibility still remains that there could be minor differences in the $\alpha$-chain carbohydrates; in the future this will be evident from mass spectrometric analysis and quantification of oligosaccharides released from the purified $\alpha$ subunits.

There are four consensus sites for glycosylation on the $\gamma$ chain. The four asparagines are (in consensus numbering): 70

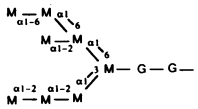
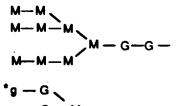
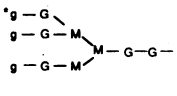
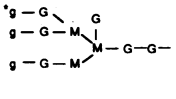
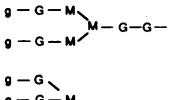
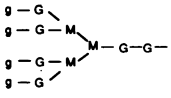
| MONOSACCHARIDE COMPOSITION | STRUCTURE | MOLECULAR WEIGHT | APPROXIMATE MOLE % |
|---|---|---|---|
| $Man_8\ GlcNAc_2$ |  | 1720 | 40 |
| $Man_9\ GlcNAc_2$ |  | 1882 | 30 |
| $Gal_3Man_3\ GlcNAc_5$ |  | 2005 | 12 |
| $Gal_3Man_3\ GlcNAc_6$ |  | 2208 | 6 |
| $Gal_2Man_3\ GlcNAc_4$ |  | 1640 | 2 |
| $Gal_4Man_3\ GlcNAc_6$ |  | 2370 | 2 |

FIG. 4. The molecular weights, monosaccharide composition, structures of oligosaccharides (as deduced by fragmentation analysis), and approximate molar percentages of the major species are recorded. M, mannose; G, *N*-acetylglucosamine; g, galactose. The molecular weights shown reflect the oligosaccharide minus the ABEE group ($M_r$, 149). Approximate mol % values were calculated from integrated peak sizes from the HPLC chromatograms. The linkage M$\alpha$1–6M as opposed to M$\alpha$1–2M was shown by methylation analysis and is in accordance with the $^1H$ NMR data of Nomoto *et al.* (23). The biantennary branch of the triantennary structures (asterisk) is shown attached to the M$\alpha$1–6M branch of the oligosaccharide. This information cannot be inferred from fragmentation data but was chosen to be in accordance with the HPLC data of Nomoto *et al.* (23).

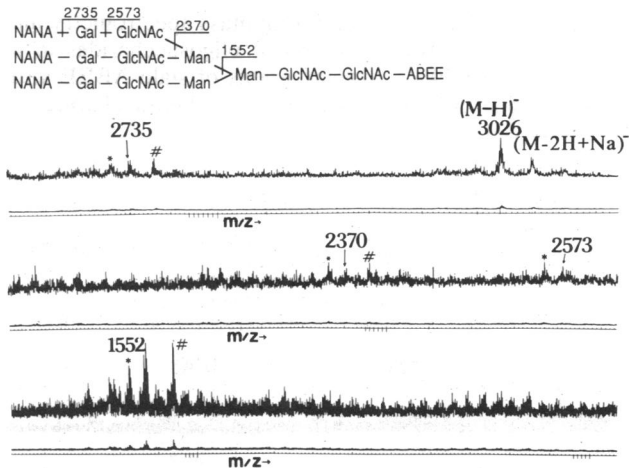



FIG. 5. The negative-ion LSIMS spectrum of a triantennary sialo-oligosaccharide derivatized with the ABEE reagent, which was obtained on ≈300 pmol (1 $\mu$g) of material. Fragmentation of glycosidic bonds is observed with charge retention at the derivatized reducing terminus. *, Species result from glycosidic bond cleavage and the loss of a water molecule from the parent molecular ion; #, species include C1 of the non-reducing terminal monosaccharide.

and 143, which lie in the soluble domain N terminal to M1; 315, which according to secondary structure predictions is located on the cytoplasmic side of M2; and 453, between MA and M4 (Fig. 1). We have established that Asn-453$_{\gamma C}$ is not glycosylated, demonstrating that there are a maximum of three oligosaccharides attached to the $\gamma$ chain. If the $\gamma$ chain does carry three oligosaccharides, as demonstrated by Anderson and Blobel (28), one of the oligosaccharides must be attached at Asn-316; this would imply that the orientation of M3 in the bilayer has to be inverted.

The direct sequencing and determination of the branched structure from fragmentation of the ABEE-derivatized oligosaccharides implies that monoclonal antibodies directed against specific oligosaccharides can now be made and used as probes for membrane proteins present in purified form at the picomolar level, to the femtomolar level in the future. It has not escaped our notice that this may be useful in localizing specific rarely expressed membrane proteins.

**AcChoR Phosphorylation.** Three endogenous protein kinases have been shown to phosphorylate specific subunits of the *Torpedo* acetylcholine receptor; namely, cAMP-dependent protein kinase, protein kinase C, and a tyrosine-specific protein kinase (reviewed in ref. 29). All of the seven possible sites for phosphorylation by these three endogenous protein kinases were located and examined in this work, and no phosphorylated peptides were detected. It is possible that these sites are labile to endogenous phosphatases, even in the continued presence of sodium fluoride and protease inhibitors.

**Advances in Structure Determination Using Mass Spectrometry.** This study of chemical and posttranslational modification of a complex multisubunit membrane protein was carried out on a total of 4 nmol of sample (1 mg of AcChoR). A combination of the protocol described here and the sensitivity of high-performance tandem sector systems with electrooptical multichannel array detection will permit sequencing of the protein, structural characterization of the posttranslational modifications, and determination of the nature and sites of covalent modifications resulting from photoaffinity experiments at the subnanomole level (20).

The mass spectrometric analyses of oligosaccharides demonstrate that the ABEE derivative is superior to the underivatized oligosaccharide, since the introduction of a hydrophobic group greatly increases the surface activity of the compound and hence the sensitivity of the analyses (21). In addition, excellent fragmentation of the oligosaccharides, predominantly at successive glycosidic linkages, is provided in the negative-ion mode (13, 14). Also, this derivatization adds only 149 Da in mass, as opposed to permethylation or peracetylation which adds $14(n+m)$ or $42(n)$ Da ($n$ is the number of free hydroxyl groups, $m$ is the number of NH-Ac groups) to the mass of an oligosaccharide. In the case of larger oligosaccharides, our method is of particular advantage, since a large increase in mass upon derivatization would move the molecular mass from the readily accessible mass range where instrument sensitivity is in the subnanomole range. ABEE derivatization also produces a homogeneous product, in contrast to the mixtures often obtained from use of permethylation or peracetylation procedures (30).

When the ABEE derivative is prepared, molecular weight and fragmentation data can readily be obtained by using 1 $\mu$g of oligosaccharide, even using conventional postacceleration and a single-slit ion multiplier system. However, this work has established that when computer-controlled mass window stepping is used with multichannel array detection, the level of sample required can be reduced by as much as 100-fold using a 4% mass window array (1000 channels). Design of ion optical system that will permit full focal plane multichannel array detection (31) will increase the sensitivity herein reported by yet at least a further order of magnitude. Such ultrahigh mass spectral sensitivities will revolutionize future investigations of the structural biology of membrane-bound glycoproteins such as growth factor receptors, which cannot be readily obtained in nanomole quantities. It will then be possible to sequence and structurally characterize proteins, membrane proteins, and their posttranslational modifications from femtomolar quantities.

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